PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	12 AUG 2005
Applicant's or agent's file reference BH-003-PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US05/09829	23 March 2005 (23.03.2005)	25 March 2004 (25.03.2004)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12Q 1/68; C12P 19/34 and US Cl.: 435/6, 91.1, 91.2

Applicant

BIOHELIX CORPORATION

1. This opinion contains indications relating to the following items:

- [X] Box No. I — Basis of the opinion
- [ ] Box No. II — Priority
- [ ] Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV — Lack of unity of invention
- [X] Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI — Certain documents cited
- [ ] Box No. VII — Certain defects in the international application
- [X] Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis(b)* that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Young J. Kim Telephone No. (571) 272-1600

Form PCT/ISA/237 (cover sheet) (January 2004)

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International application No.
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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing

      ☐ contained in international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 9-11, 21, and 22	YES
	Claims 1-8 and 12-20	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-22	NO
Industrial applicability (IA)	Claims 1-22	YES
	Claims NONE	NO

2. Citations and explanations:

Claims 1-8 and 12-20 lack novelty under PCT Article 33(2) as being anticipated by Auerbach (U.S. Patent No. 5,354,668, issued October 11, 1994).

Auerbach discloses a method of amplifying a target nucleic acid, in an embodiment circular nucleic acid (Figure 8B, column 14, lines 34-37) , wherein said method involves the use of a helicase (column 11, line 44), anticipating claims 1-6, 8, 12-14, and 17-20.

With regard to claim 7, the method employs several types of DNA polymerases, such as Taq, T7, T4, and T5 polymerase (column 7, lines 40-44).

With regard to claims 15 and 16, the process is disclosed as being isothermal (column 14, line 37).

Therefore, Auerbach anticipates the invention as claimed.

Claims 9-11, 21, and 22 lack an inventive step under PCT Article 33(3) as being obvious Auerbach (U.S. Patent No. 5,354,668, issued October 11, 1994) in view of Mendez et al. (BioEssays, 2003, vol. 25, pages 1158-1167).

Auerbach discloses a method of amplifying a target nucleic acid, in an embodiment circular nucleic acid (Figure 8B, column 14, lines 34-37) , wherein said method involves the use of a helicase (column 11, line 44).
The method employs several types of DNA polymerases, such as Taq, T7, T4, and T5 polymerase (column 7, lines 40-44).
The method, in an embodiment, is disclosed as being isothermal (column 14, line 37).

Auerbach do not explicitly disclose the type of helicase that could be employed in their method of amplification.

Mendez et al. disclose a method of amplification involving hexameric helicases (page 1163, 1st column, 2nd paragraph).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ any of well-known helicases in the method of amplification involving polymerase, for the advantage offered by the use of helicases, the advantage of which has been clearly discussed by Auerbach:
"For instatnce, as indicated above, the addition of topoisomerase, helicases, gyrases or single-stranded nucleic acid binding proteins...may be used to increase the strand displacement rate of a DNA polymerase, or may allow the use of a DNA polymerase that might not ordinarily give substantial amplification" (column 11, lines 43-49).

Thus, one of ordinary skill in the art, at the time the invention was made would have been clearly motivated to combine the use of a helicase with a DNA polymerase in the method of amplification, for the explicit benefit of facilitating the processivity of said DNA polymerase, resulting in the enhancement of the amplification reaction with a reasonable expectation of success.

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Claim 1 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim is indefinite for the following reason(s):
Claim 1 is indefinite for reciting the term, "helicase preparation," because it is unclear what this preparation contains.